Airway Epithelial Cell Responses to Ozone Injury

George D. Leikauf,[1] Leigh G. Simpson,[1] Jeffrey Santrock,[2] Qiyu Zhao,[1] Joan Abbinante-Nissen,[1] Shaoying Zhou,[1] and Kevin E. Driscoll[3]

[1]Pulmonary Cell Biology Laboratory, Departments of Environmental Health, Molecular and Cellular Physiology, and Medicine, University of Cincinnati Medical Center, Cincinnati, Ohio; [2]Biomedical Science Department, General Motors Research and Environmental Staff, Warren, Michigan; [3]Health and Safety Division, Procter and Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio

The airway epithelial cell is an important target in ozone injury. Once activated, the airway epithelium responds in three phases. The initial, or immediate phase, involves activation of constitutive cells, often through direct covalent interactions including the formation of secondary ozonolysis products—hydroxyhydroperoxides, aldehydes, and hydrogen peroxide. Recently, we found hydroxyhydroperoxides to be potent agonists of bioactive eicosanoid formation by human airway epithelial cells in culture. Other probable immediate events include activation and inactivation of enzymes present on the epithelial surface (e.g., neutral endopeptidase). During the next 2 to 24 hr, or early phase, epithelial cells respond by synthesis and release of chemotactic factors, including chemokines—macrophage inflammatory protein-2, RANTES, and interleukin-8. Infiltrating leukocytes during this period also release elastase, an important agonist of epithelial cell mucus secretion and additional chemokine formation. The third (late) phase of ozone injury is characterized by eosinophil or monocyte infiltration. Cytokine expression leads to alteration of structural protein synthesis, with increases in fibronectin evident by *in situ* hybridization. Synthesis of epithelial antiproteases, e.g., secretory leukocyte protease inhibitor, may also increase locally 24 to 48 hr after elastase concentrations become excessive. Thus, the epithelium is not merely a passive barrier to ozone injury but has a dynamic role in directing the migration, activating, and then counteracting inflammatory cells. Through these complex interactions, epithelial cells can be viewed as the initiators (alpha) and the receptors (omega) of ozone-induced airway disease. — Environ Health Perspect 103 (Suppl 2):91–95 (1995)

Key words: air pollution, asthma, bronchitis, cytokines, inflammation, eicosanoids, aldehydes, hydroxyhydroperoxides, hydrogen peroxide

Introduction

Concerns continue to increase about whether the current ambient air quality standard for ozone (120 ppb) provides an adequate margin of safety for the U.S. population. Several clinical (*1–4*) and epidemiological (*5–7*) studies, some of which are presented in detail in this paper, have documented significant decrements in pulmonary airflow in persons exposed to ≤120 ppb (Table 1). Such effects are more readily observed in exercising individuals when exposures are extended (>4 hr) (*8*).

In related studies, another consistent finding is an increase in inflammatory cells in bronchoalveolar lavage fluid obtained after ≤120 ppb ozone exposure (*1,3,4*). Increases in inflammatory mediators (bioactive lipids and cytokines) and protease (elastase) accompany this change (Figure 1). The cellular sources of these mediators in bronchoalveolar lavage fluid from proximal conducting airway and distal lung regions are uncertain.

Although the bronchoalveolar junction is considered the primary site of ozone attack in the lung (*9*), another important target site is the airway epithelium (*10*). Previous analyses of regional ozone deposition and absorption (*11,12*) suggest that exposure to high concentrations of ozone could result in deposition sufficient to induce epithelial damage (histological changes). Such ozone exposure can alter the maintenance of epithelial barrier (*13–15*) and particle clearance function (*16*). However, at lower concentrations (i.e., ~100 ppb), ozone deposition in the airways is likely to be less, and health effects are thus more difficult to uncover experimentally. Nonetheless, the apparent changes in airway function (e.g., decreased forced expiratory volume [$FEV_{1.0}$]) argues that airway perturbation (perhaps at a level undetected by conventional histology) must occur even at low level exposure.

Subtle injury to epithelial cells may play an active role in systemic airway responses following low-level ozone exposure (*5,17,18*). In evaluating these effects, it is important to consider both constitutive functions and elicited defense mechanisms of the epithelium (Table 2). Ozone may influence cellular function directly by chemical modification of molecular constituents of the cell membrane or indirectly by the interaction of its primary reaction products

Table 1. Pulmonary response to ozone.

Decreased forced expiratory volume –1.0 ($FEV_{1.0}$)[a]
Airway hyperreactivity[a]
Airway inflammation[a]
Decreased athletic performance[a]
Increased cough and symptoms (substernal discomfort, etc.)[a]
Altered tracheobronchial clearance
Increased epithelial permeability

[a]Occur at or below current National Air Quality Standard of 120 ppb.

This is an updated and expanded version of an article that was presented at the Environmental and Occupational Health Sciences Institute Symposium on Ozone Air Quality and Health Effects held 27–29 May 1992 in Piscataway, New Jersey.

The authors thank Edwin Donnelly and Thomas Bordner for technical assistance and Danny Edmonds for manuscript preparation. This study was supported in part by grants R01-ES06562, R01-ES06677, and P30-ES06096 from the National Institute of Environmental Health Sciences, the Health Effects Institute, Agreement 90-13, an organization jointly funded by the U. S. Environmental Protection Agency (Assistance Agreement X-812059) and automotive manufacturers; the Electric Power Research Institute grant RP2155-03; the Center for Indoor Air Research grant 93-08A; and the Procter and Gamble Company, UAARP-21314.

The contents of this article do not necessarily reflect the views of the Health Effects Institute nor the policies of the U.S. Environmental Protection Agency or automotive manufacturers.

Address correspondence to Dr. George D. Leikauf, Pulmonary Cell Biology Laboratory, Department of Environmental Health, University of Cincinnati, PO Box 670056, Cincinnati, OH 45267-0056. Telephone (513) 558-0039. Fax (513) 558-4397.

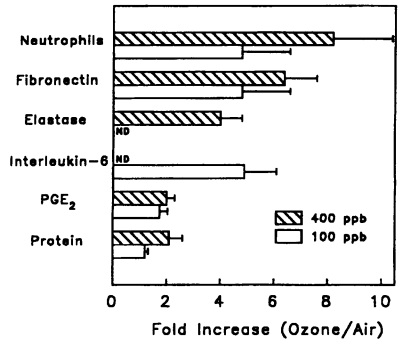

Figure 1. Abbreviations: ND, not done, PGE_2, prostaglandin E_2. Increases in inflammatory cells, proteins, and mediators measured in bronchoalveolar lavage fluid obtained from persons exposed for 6.6 hr to 400 ppb (hatched bars) or 100 ppb (open bars) ozone. Adapted from Delvin et al. (*4*).

Table 2. Constitutive and elicited defense mechanisms of the airway epithelium.

A. Constitutive function
1. Mucus synthesis and secretion
2. Ciliary clearance
3. Ion transport and fluid movement
4. Biotransformation
a. Neutral endopeptidase
b. Cytochrome P450 (1A1)
5. Antiinflammatory activity
a. Eicosanoids (PGE_2)
B. Elicited defense mechanisms
1. Antiprotease
a. Secretory leukocyte protease inhibitor
2. Antioxidant
a. Superoxide dismutase (SoxR)
b. Catalase (OxyR)
3. Antimicrobial
a. Lysozyme

Table 3. Time-course of responses to injury in the airway epithelium.

A. Immediate responses (0–2 hr) (constitutive cell activation)
1. Chemical reaction with membrane
2. Biotransformation
a. Cytochrome P450
b. Neutral endopeptidase
3. Bioactive lipid release
a. Eicosanoids
b. Platelet-activating factor[a]
4. Sensory neuro-reflex pathways[a]
B. Early responses (2–24 hr) (neutrophil infiltration)
1. Bioactive lipid release
2. Proinflammatory mediator release (neutrophil chemotaxins)
3. Antiinflammatory mediator release (prostanoids)
4. Cytokine release
a. Tumor necrosis factor-α[a]
b. Interleukins (IL-1, IL-6, IL-8, etc.)
5. Protease release
a. Elastase[a]
b. Cathepsin G[a]
C. Late responses (12–24 hr) (eosinophil/monocyte infiltration
1. Bioactive lipid release
a. Increases in proinflammatory (monocyte chemotaxins)
b. Decreases in anti-inflammatory (prostanoids)
2. Cytokine release
a. Granulocyte, macrophage-colony stimulating factor (eosinophil proliferation)
b. Transforming growth factor-β
c. Platelet-derived growth factor[a]
3. Intercellular adhesion molecules
4. Structural proteins synthesis
a. Collagen
b. Fibronectin
5. Major basic protein (from eosinophilic infiltration)[a]
6. Antiprotease synthesis and release
a. Secretory leukocyte protease inhibitor
7. Antioxidants synthesis
a. Superoxide dismutase
b. Catalase
c. Glutathione

[a]Not produced (or yet not confirmed) by human airway epithelial cells. All others can be synthesized by and released from airway epithelial cells.

with the cell. Although the anatomical, cellular, and biochemical sites of reaction of ozone in the lungs are not known, it is generally believed that ozone does not penetrate further than the airway epithelium because of its high reactivity (*19*). This would confine ozone to react with substances in the extracellular fluid in the airway lumen and in the apical plasma membrane of the epithelial cell. The resultant ozonolysis products are generally more stable than ozone itself and can diffuse farther into the airway epithelium, where they may activate one or more mediator cascades and thereby elicit a variety of cellular responses. Here, we divide the likely events associated with ozone injury to the airway epithelium into immediate, early, and late phases (Table 3).

Immediate Phase

The immediate phase occurs during (or within 2 hr after) exposure and is characterized by activation of constitutive cells in the airway epithelium. Because it is a highly reactant irritant (*20*), ozone's attack may be limited to components of the respiratory tract fluid and the apical plasma membrane, with little unreacted ozone penetrating into the cytosol (*19*). Examination of ozone reactions with amino acids (*21,22*), proteins (*23–28*), unsaturated fatty acids (*29–31*), and phospholipids containing fatty acids (*32–35*) *in vitro* indicates that ozone is likely to react with cysteine, methionine, tyrosine, tryptophan, and histidine residues in proteins and carbon–carbon double bonds of unsaturated fatty acids in membrane lipids (Figure 2). The latter reactions yield hydrogen peroxide, aliphatic and allelic aldehydes and hydroxyhydroperoxides of various chain lengths (*32,35*).

Recently, we have investigated the ability of selected ozonolysis products of fatty acids to activate membrane phospholipases in human airway epithelial cells in culture (*36*). Compounds with longer chain lengths were more potent than compounds of shorter chain length ($C_9 > C_6 > C_3$), and hydroxyhydroperoxides were more potent than corresponding aldehydes or hydrogen peroxide in initiating eicosanoid release from human airway epithelial cells. Significant effects were observed at concentrations of ≥3 μM 1-hydroxy-1-nonanehydroperoxide.

To place these findings into perspective with regard to *in vivo* exposure concentration, we estimate the rate of hydroxyhydroperoxide production at the airway surface based on available estimates of ozone deposition in the lung. Many difficulties are associated with such an estimate, so an initial point is to apply the dosimetric model developed by Miller and Overton (*11,12*) that predicts a dose of up to approximately 2 pg O_3 deposited/cm^2 tissue × min per μg ambient O_3/m^3 air. This value can be higher or lower, depending on breathing rate and site of regional deposition, respectively. At an ambient concentration of 120 ppb (2.4×10^8 pg/m^3 air: the current ambient air quality standard), the rate of adsorption of ozone by the airway epithelium would be 10 pmole/cm^2 × min (480 pg/cm^2 × min ÷ 48 pg/pmole) From the stoichiometry of the reaction, we know that 1 molecule of hydroxyhydroperoxide will be produced from every molecule of ozone that reacts with a carbon–carbon double bond in an unsaturated fatty acid. If we limit the amount of ozone that reacts with unsaturated fatty acids to 20% of the absorbed dose, then the hydroxyhydroperoxides formation rate would be approximately 2 pmole/cm^2 × min. Assuming these products remain within a 10 μm layer on the airway surface, the concentration of hydroxyhydroperoxide is approximately equal to the lowest dose (3 μM) that elicited [^{3}H]-activity release from airway epithelial cells.

These findings suggest that secondary ozonolysis products also have biological activity. Because they are more stable than ozone, these compounds can move from their formation site and react within the cytoplasm, activating other enzymes (e.g., cyclooxygenase). Thus, inhaled ozone can generate chemical intermediates that can react with and activate epithelial cells.

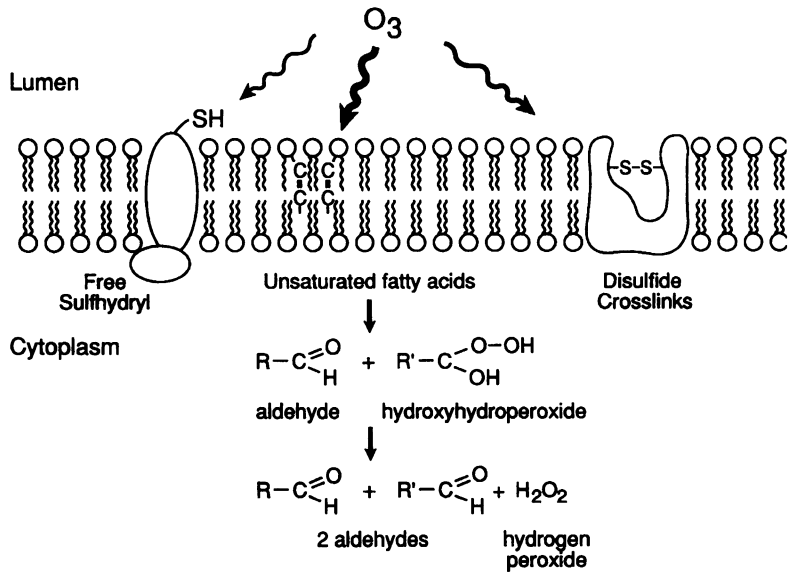

Figure 2. Possible reaction sites of ozone with cellular macromolecules. The three preferred sites are free sulfhydryl groups in membrane proteins, carbon–carbon double bonds in unsaturated fatty acids, and disulfide crosslinks in membrane proteins. Ozonolysis of membrane fatty acids leads to the formation of an aldehyde and a hydroxyhydroperoxide, one retained in the membrane the other free to exist the membrane and react with cytoplasmic constituents. When a hydroxyhydroperoxide is formed, it subsequently reacts in the presence of water to form a second aldehyde and hydrogen peroxide (H_2O_2). Thus, the net reactions yield two aldehydes and H_2O_2.

In addition to immediate activation of phospholipases (responsible for arachidonic acid release and subsequent metabolism by specific pathways), ozone could possibly alter other enzymes responsible for biotransformation capacity within the epithelium. Again, this could result from direct ozone reactions with amino acid in membrane-bound proteins (*24,28*) or through the action of reactive intermediates (such as hydroxyhydroperoxides) that can act at sites distant (cytoplasmic, nucleus) to the site of initial ozonolysis (*35*). Thus both surface and nonmembrane-bound proteins and enzymes could be activated or inactivate by ozone exposure.

The biological consequences of other inhaled materials, when absorbed subsequently, can also be influenced by this process because they can be activated by epithelial enzymes (e.g., aromatic hydrocarbon by cytochrome P_{450}) or inactivated by other enzymes, (e.g., aldehydes by aldehyde dehydrogenases). Thus, alteration in constitutive "metabolic" functions within the epithelial cell may affect local response to subsequent stimuli. Experimental details are yet to be obtained with ozone in this area, this may be a fertile subject for further research.

An example of inactivation/activation of epithelial enzymes has been clarified following toluene diisocyanate inhalation. This irritant can inactivate neutral endopeptidase, contained on the plasma membrane surface of airway basal cells. Loss of neutral endopeptidase can in turn heighten airway smooth muscle reactivity to substance P and other neuropeptides (*37*). Similarly, loss of neutral endopeptidase activity caused by toluene diisocyanate may influence subsequent responses to irritants such as compounds that induce mucosal edema or mucus gland secretion (through mechanisms involving retrograde neurotransmitter release from sensory neurons). Data on ozone's effects on this important enzyme are preliminary (*38,39*), but recent evidence suggest that neuropeptide release and inactivation of neutral endopeptidase can also occur following ozone exposure.

Early Phase

During 2 to 24 hr after acute injury, the next (early) phase is initiated and characterized by infiltration of polymorphonuclear leukocytes (predominately neutrophils). Epithelial cells, along with other resident cells, can synthesize and release chemotactic factors thereby directing migration and activation of neutrophils (*17,40,41*). This, in part, results from augmented bioactive lipid mediator release from both resident and migratory cells, with elevations present in pro-inflammatory eicosanoid (e.g., leukotriene B_4 or prostaglandin $F_{2\alpha}$). As noted previously, ozone activates eicosanoid metabolism in airway epithelial cells (*17,36*).

Additional elevations of cytokine concentrations, demonstrated to be derived from airway epithelial cells, may also play an important role in orchestrating this early response to ozone. Elevated synthesis and release of interleukins (IL) and possibly tumor necrosis factor (TNF_α) during this early period are thought to activate the subsequent release of secondary mediators. For example, IL-1 and TNF_α may be responsible for secondary IL-6 and IL-8 expression. Recently, we have begun to examine the effects of ozone inhalation on macrophage inflammatory protein-2 (MIP-2), a chemokine, in mouse lung (42). These results indicate that MIP-2 mRNA transcript levels are increased (as determined by reverse transcription–polymerase chain reaction) at doses that produce increases in neutrophils in lavage fluid. Increases in MIP-2 transcript levels also precede the neutrophil infiltration. Our initial conclusion is that ozone-induced chemokine (including MIP-2 and related proteins, IL-8, RANTES, etc.) expression has a clearer association with neutrophil infiltration than does chemotactic eicosanoid (leukotriene B_4 or 15-diHETE) formation.

Once leukocytes are present, the profile of eicosanoid metabolites within the epithelium also may change through transcellular eicosanoid metabolism. For example, leukotriene A_4 (LTA_4) released from activated neutrophils can be subsequently metabolized to leukotriene B_4 (LTB_4) by adjacent cells. Recently, we have begun examining transcellular metabolism with airway cells and neutrophils stimulated with Ca-ionophore, a stimulus of phospholipase. Leukotriene B_4 production is increased when cells are cocultured. Since ozone also stimulates phospholipase, it is likely that it could also affect LTA_4-LTB_4 co-metabolism.

Finally, during this period, elastase and cathepsin G can be released from incoming cells. Neutrophil elastase is clearly elevated in bronchoalveolar lavage in persons exposed to ozone (Figure 1). Elastase is a potent agonist for mucus secretion (*43*) and chemokine formation (*41*).

Late Phase

The third phase of ozone injury is characterized by a period of eosinophil (important in asthma) or monocyte (important in bronchitis) infiltration. Clear alterations in DNA transcription, steady-state mRNA levels, and protein synthesis are likely to occur during this period. Proinflammatory

eicosanoid (and possibly cytokine) synthesis in the epithelium continues during this period, with release of additional monocyte chemotaxins (*44*), and possibly granulocyte, macrophage-colony stimulating factor (*45,46*) or transforming growth factor-β (*47–49*).

During this time, factors like transforming growth factor-β may markedly enhance fibronectin mRNA expression. In human airway epithelial cells, as evidenced by cells in culture, TGF_β-induced fibronectin mRNA expression can increase with a time course consistent with a late phase response (maximal levels occur after 18 to 24 hr). Synthesis of other structural proteins (important in tissue repair), antiproteases (including secretory leukocyte protease inhibitor) (*50*), and antioxidants (superoxide dismutase, catalase and glutathione reductase) (*51*) also increase during this period. The loss of anti-inflammatory eicosanoids through suicidal, cyclooxygenase inactivation may aggravate leukocyte activation, protease release, and tissue damage. Clearly several of these possibilities merit further investigation.

Summary

Inhalation of ozone in the past has been associated with airway dysfunction marked by physiologic and histological change. These changes are typically noted only after high level exposure. More subtle changes have been noted in recent years which may or may not be associated with histological changes. Through a clearer understanding of ozone's preferred targets and the role of subtle biochemical changes in the airway epithelium, the early events in a host-initiated cascade have been uncovered. At present, aspects of the above discussion are still speculative due to a lack of experimental details. (For example, does the increase in MIP-2 observed in mouse lung occur in humans?) One purpose of this presentation is to present future research directions that may increase our knowledge of the cellular and subcellular mechanisms responsible for ozone toxicity.

Nonetheless, at present we can conclude that epithelium is not passive in airway injury/response to ozone. It has an active role in directing the migration of inflammatory cells and in the release and co-metabolism of inflammatory mediators. Thus, it is partially responsible for changes in the microenvironment surrounding the epithelial cells. Ultimately, these changes can alter the phenotype of the epithelial cell population, possibly leading to persistent changes in cell metabolism (e.g., loss of neutral endopeptidase) or in mucin-producing cell types important in hypersecretory diseases (bronchitis). In this way, epithelial cells can be viewed as the alpha (initiator) and the omega (receptor) cell in ozone-induced airway disease.

REFERENCES

1. Seltzer J, Bigby BG, Stulbarg M, Holtzman MJ, Nadel JA, Ueki IF, Leikauf GD, Goetzl EJ, Boushey HA. O_3-induced change in bronchial reactivity to methacholine and airway inflammation in humans. J Appl Physiol 60:1321–1326 (1986).
2. Horstman DH, Folinsbee LJ, Ives PJ, Abdul-Salaams S, McDonnell WF. Ozone concentration and pulmonary response relationships for 6.6-hour exposures with five hours of moderate exercise to 0.08, 0.10, and 0.12 ppm. Am Rev Respir Dis 142:1158–1163 (1990).
3. Koren HS, Devlin RB, Graham DE, Mann R, McGee MP, Horstman DH, Kozumbo WJ, Becker S, House DE, McDonnell WF, Bromberg PA. Ozone-induced inflammation in the lower airways of human subjects. Am Rev Respir Dis 139:407–415 (1989).
4. Devlin RB, McDonnell WF, Mann R, Becker S, House DE, Schreinemachers D, Koren HS. Exposure of humans to ambient levels of ozone for 6.6 hours causes cellular and biochemical changes in the lung. Am J Respir Cell Mol Biol 4:72–81 (1991).
5. Lippmann M. Health effects of ozone. J Air Pollution Control Assoc 39:692–695 (1989).
6. Schwartz J. Air pollution and the duration of acute respiratory symptoms. Arch Environ Health 47:116–122 (1992).
7. Kryzanowski M, Quackenboss JJ, Lebowitz MD. Relation of peak expiratory flow rates and symptoms to ambient ozone. Arch Environ Health 47:107–115 (1992).
8. Folinsbee LJ, McDonnell WF, Horstman DH. Pulmonary function and symptom responses after 6.6 hours to 0.12 ppm ozone with moderate exercise. J Air Pollution Control Assoc 38:28–35 (1988).
9. Barry BE, Mercer RR, Miller FJ, Crapo JD. Effects of inhalation of 0.25 ppm ozone on the terminal bronchioles of juvenile and adult rats. Exp Lung Res 14:225–237 (1988).
10. Hyde DM, Hubbard WC, Wong V, Wu R, Pinkerton K, Plopper CG. Ozone-induced acute tracheobronchial epithelial injury: relationship to granulocyte emigration in the lung. Am J Respir Cell Mol Biol 6:481–497 (1992).
11. Miller FJ, Menzel DB, Coffin DL. Similarity between man and laboratory animals in regional pulmonary deposition of ozone. Environ Res 17:84–101 (1978).
12. Miller FJ, Overton JH Jr, Jaskot RH, Menzel DB. A model of the regional uptake of gaseous pollutants in the lung. Toxicol Appl Pharmacol 79:11–27 (1985).
13. Bhalla DK, Crocker TT. Tracheal permeability of rats exposed to ozone. Am Rev Respir Dis 134:572–579 (1986).
14. Young C, Bhalla DK. Time course of permeability changes and PMN flux in rat trachea following O_3 exposure. Fundam Appl Toxicol 18:175–180 (1992).
15. Kleeberger SR, Hudak BB. Acute ozone-induced change in airway permeability: role of infiltrating leukocytes. J Appl Physiol 72:670–676 (1992).
16. Foster WM, Costa DL, Langenback EG. Ozone exposure alters tracheobronchial mucociliary function in humans. J Appl Physiol 63:996–1002 (1987).
17. Leikauf GD, Driscoll KE, Wey HE. Ozone-induced augmentation of eicosanoid metabolism in cultured epithelial cells from bovine trachea. Am Rev Respir Dis 137:435–442(1988).
18. Leikauf GD, Driscoll KE. Cellular approaches in respiratory tract toxicology. In: Toxicology of the Lung (Gardner DE, Crapo JD, McClellan RO, eds). New York:Raven Press, 1993;335–370.
19. Pryor WA. How far does ozone penetrate into the pulmonary air/tissue boundary before it reacts? Free Radic Biol Med 12:83–88 (1992).
20. Mustafa MG. Biochemical basis of ozone toxicity. Free Radic Biol Med 9:245–265 (1990).
21. Mudd JB, Leavitt R, Ongun A, McManus TT. Reaction of ozone with amino acids and proteins. Atmos Environ 3:669–682 (1969).
22. Pryor WA, Giamalva DH, Church DF. Kinetics of ozonation. II. Amino acids and model compounds in water and comparisons to rates in nonpolar solvents. J Am Chem Soc 106:7094–7100 (1984).
23. Freeman BA, Sharman MC, Mudd JB. Reaction of ozone with phospholipid vesicles and human erythrocytes ghosts. Arch Biochem Biophys 197:264–272 (1979).
24. Freeman BA, Mudd JB. Reactions of ozone with sulfhydryls of human erythrocytes. Arch Biochem Biophys 208:212–220 (1981).

25. Sharman MC, Mudd JB. Ozone inactivation of anti-elastase activity of chicken ovoinhibitor and human alpha-1-proteinase inhibition. Biochem Biophys Res Commun 102:640–645 (1981).
26. Peters RE, Mudd JB. Inhibition by ozone of the acylation of glycerol-3-phosphate in mitochondria and microsomes from rat lung. Arch Biochem Biophys 216:34–41 (1982).
27. Dooley MM, Mudd JB. Reaction of ozone with lysozyme under different exposure conditions. Arch Biochem Biophys 281:459–471 (1981).
28. Knight KL, Mudd JB. The reaction of ozone with glyceraldehyde-3-phosphate dehydrogenase. Arch Biochem Biophys 229:259–269 (1984).
29. Roehm JN, Hadley JC, Menzel DB. Oxidation of unsaturated fatty acids by ozone and nitrogen dioxide: a common mechanism of action. Arch Environ Health 23:142–148 (1971).
30. Giamalva DH, Church DF, Pryor WA. A comparison of the rates of ozonation of biological antioxidants and oleate and linoleate esters. Biochem Biophys Res Commun 133:773–779 (1985).
31. Pryor WA, Das B, Church DF. The ozonation of unsaturated fatty acids: aldehydes and hydrogen peroxide as products and possible mediators of ozone toxicity. Chem Res Toxicol 4:341–348 (1991).
32. Tiege B, McManus TT, Mudd JB. Reaction of ozone with phosphatidylcholine liposomes and the lytic effects of products on red blood cells. Chem Phys Lipids 12:153–171 (1974).
33. Uemura K, Hara A, Taketomi T. Chemical and hemolytic properties of sphingolipids modified by ozonolysis and reduction. J Biochem (Tokyo) 79:1253–1261 (1976).
34. Butterman J, Chan PC, Kesner L. Generation of hemolytic activity in ozone-treated phosphatidylcholine. Environ Res 42:406–414 (1987).
35. Santrock J, Gorski RA, O'Gara JF. Products and mechanisms of the reaction of ozone with phospholipids in unilamellar phospholipid vesicles. Chem Res Toxicol 5:134–141 (1992).
36. Leikauf GD, Zhao Q, Zhou S, Santrock J. Ozonolysis products of membrane fatty acids activate eicosanoid metabolism in human airway epithelial cells. Am J Respir Cell Mol Biol 9:594–602 (1993).
37. Barnes PJ, Baranink JN, Belvisi MG. Neuropeptides in the respiratory tract. Am Rev Respir Dis 144:1187–1198 (1991).
38. Yeadon M, Wilkinson D, Darley-Usmar V, O'Leary SJ, Payne AN. Mechanisms contributing to ozone-induced bronchial hyperreactivity in guinea pigs. Pulm Pharmacol 5:39–50 (1992).
39. Norris AA, Leeson ME, Jackson DM, Holroyde MC. Modulation of neurogenic inflammation in rat trachea. Pulm Pharmacol 3:180–184(1990).
40. Koyama S, Rennard SI, Leikauf GD, Shoji S, Von Essen S, Claasen K, Robbins RA. Endotoxin stimulates bronchial epithelial cells to release chemotactic factors for neutrophils. J Immunol 147:4293–4301 (1991).
41. Nakamura H, Yoshimura K, McElvaney NG, Crystal RG. Neutrophil elastase in respiratory epithelial lining fluid of individuals with cystic fibrosis induces interleukin-8 gene expression in a human bronchial epithelial cell line. J Clin Invest 89:1478–1484 (1992).
42. Driscoll KE, Simpson LG, Carter J, Hassenbein D, Leikauf GD. Ozone inhalation stimulates expression of the neutrophil chemotactic peptide, MIP-2. Toxicol Appl Pharmacol 119:306–309 (1993).
43. Sommerhoff CP, Nadel JA, Basbaum CB, Caughey GH. Neutrophil elastase and cathepsin G stimulate secretion from cultured bovine airway gland serous cells. J Clin Invest 85:682–689 (1990).
44. Koyama S, Rennard SI, Leikauf GD, Robbins RA. Bronchial epithelial cells release monocyte chemotactic activity in response to smoke and endotoxin. J Immunol 147:972–979 (1991).
45. Ohostahi T, Vancheri C, Cox G, Gauldie J, Dolovich J, Denburg JA, Jordana M. Monocyte-macrophage differentiation induced by human upper airway epithelial cells. Am J Respir Cell Mol Biol 4:255–263 (1991).
46. Soloperto M, Mattoso VL, Fasoli A, Mattoli S. A bronchial epithelial cell-derived factor in asthma that promotes eosinophil activation and survival as GM-CSF. Am J Physiol 260:L530-L538 (1991).
47. Pelton RW, Moses HL. The beta-type transforming growth factor. Mediators of cell regulation in the lung. Am Rev Respir Dis 142:531–535 (1990).
48. Khalil N, O'Connor RN, Unruh HW, Warren PW, Flanders KC, Kemp A, Bereznay OH, Greenberg AH. Increased production and immunohistochemical localization of transforming growth factor-beta in idiopathic pulmonary fibrosis. Am J Respir Cell Mol Biol 5:155–162 (1991).
49. Schmid P, Cox D, Bilbe G, Maier R, McMaster GK. Differential expression of TGF beta 1, beta 2 and beta 3 genes during mouse embryogenesis. Development 111:117–130 (1991).
50. Abbinante-Nissen JM, Simpson LG, Leikauf GD. Neutrophil elastase increases secretory leukocyte protease inhibitor transcript levels in airway epithelial cells. Lung Cell Mol Physiol 265:L286–L292 (1993).
51. Rahman I, Massaro D. Endotoxin treatment protects rats against ozone-induced lung edema: with evidence for the role of manganese superoxide dismutase. Toxicol Appl Pharmacol 113:13–18 (1992).